PATENT
Attorney Docket No. 067425-5001-US
Former Docket No. RADO-001/02US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

BEDNARSKI *et al.*

Application No. 10/681,855

Filed: October 7, 2003

For: X-NITRO COMPOUNDS, PHARMACEUTICAL COMPOSITIONS THEREOF AND USES THEREOF

Examiner: ANDERSON, James D.

Art Unit: 1614 Conf. No.: 7135

CERTIFICATE OF ELECTRONIC TRANSMISSION UNDER 37 C.F.R. 1.8

I hereby certify that this correspondence, including listed enclosures, is being electronically transmitted to the United States Patent and Trademark Office in accordance with 37 C.F.R. 1.6(a)(4) on:

Dated: June 12, 2008

Signed: Jennifer C. Black

DECLARATION UNDER 37 CFR 1.132

I Susan J. Knox hereby declare and state as follows:

1. I am a co-inventor of the above identified application

2. Attached as Exhibit 1 is my curriculum vitae. I consider myself to be an expert in radiation oncology, tumor biology, radiation biology and radiosensitizers.

3. I have reviewed: (1) the above identified patent application; and (2) the Office Action mailed on September 20, 2007.

4. Solid tumors contain regions that are both well oxygenated (normoxic) and hypoxic (low oxygen concentration). Treatment of both normoxic, and particularly hypoxic tumor cells, presents a difficult challenge. Normoxic tissues and tumors are generally relatively well vascularized and/or in relatively close proximity to vessels, from which oxygen diffuses. Oxygen is required for the formation of toxic reactive oxygen species following irradiation. Hypoxic tumor cells are far more resistant to radiation than normoxic tumor cells because of the low oxygen concentration in hypoxic areas of tumors.

5. There are two forms of tumor hypoxia. Chronic, diffusion-limited hypoxia exists because of the limited range of oxygen diffusion from capillaries. Acute, perfusion-limited hypoxia results from transient closure or blockage of tumor vessels, resulting in rapid onset of acute hypoxia in tumor cells in the vicinity of the closed vessels. Some tumor vessels open and close over time. This is a dynamic process that creates multiple areas of transient hypoxia, on a background of chronic hypoxia in other areas of the tumor. Staining of hypoxic cells from tumors demonstrates scattered foci of hypoxia throughout tumors, with or without a necrotic core region.

6. Hypoxic cells are resistant to ionizing radiation, and may also be resistant to many chemotherapeutic drugs. Furthermore, gene expression profiles of hypoxic cells strongly suggest that hypoxic cells have properties consistent with a higher propensity to metastasize than aerobic cells. Accordingly, the treatment of hypoxic tumor cells is an important goal in cancer treatment.

7. There is a well known relationship between oxygen concentration and radiosensitivity. Oxygen is important for the killing of tumor cells by radiation because radiation interacts with oxygen to form reactive oxygen species which are important mediators of the effects of radiation (e.g. peroxide formation in important biomolecules, such as DNA). Importantly, clinical data supports the importance of oxygen concentration in tumors for radiation treatment. For example, it is well known that patients with some tumor types (e.g. cervix, head and neck cancer) that are treated with radiation do significantly less well if they have relatively hypoxic tumors.

8. As shown in Exhibit 2, ABDNAZ is unique in that it is activated by bio-reduction to decompose (such as in the reduced state of tumors and hypoxic cells) and release therapeutic radical species, which after further decomposition, result in nontoxic byproducts. Radical species are also produced in the presence of ionizing radiation via a different mechanism than bioreduction as shown in Exhibit 3. Such radical species can further react with oxygen and water to form the common therapeutic radical species that result in conventional ROS mediated cell death.

9. Exhibit 4 is a schematic diagram of a tumor, showing areas of hypoxia, in which ABDNAZ would be activated by bioreduction. The left panel of Exhibit 5 shows data from clonogenic assays under hypoxic and normoxic conditions where survival (log scale) is plotted on the Y axis as a function of ABDNAZ concentration on the X axis. At clinically relevant doses, ABDNAZ resulted in approximately 1.5 logs more cell killing in hypoxic conditions (created using a hypoxia chamber) compared to normoxia.

10. At the time of the filing of this patent application, Tirapazamine (TPZ) was the most promising potential radiosensitizer in clinical development. A direct comparison of ABDNAZ to TPZ in a clonogenic assay is shown in the panel on the right in Exhibit 5. As can be seen, ABDNAZ was significantly more potent as a hypoxic cytotoxin than TPZ. TPZ has significant toxicity and is no longer in clinical development.

11. Based on the foregoing, it is my opinion that high energy nitro containing compounds, such as ABDNAZ, have a high probability of being used successfully in human clinical trials to treat tumors, containing both normoxic and hypoxic tumor cells, either alone or in combination with radiation therapy.

12. I an aware that willful false statements and the like are punishable by fine or imprisonment or both (18 USC 1001), and may jeopardize the validity of the patent application or any patent issuing thereon.

Date: 6/12/08 Susan J. Knox
Dr. Susan J. Knox

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Knox, Susan J.	Associate Professor, Radiation Oncology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of California, Berkeley, CA	A.B.	1974	Genetics
University of California, Davis, CA	Ph.D.	1980	Microbiology
Stanford Univ. School of Medicine, Stanford, CA	M.D.	1985	Medicine

A. Positions and Honors

Positions and Employment

6/80-6/81	Post-doctoral Research Immunologist, Laboratory for Energy-Related Health Research, University of California, Davis
6/85-6/86	Internship, Internal Medicine, University of California, Davis Medical Center,
7/87 -6/89	Post-doctoral Fellowship, Departments of Medicine (Oncology) and Radiation Oncology, Stanford University School of Medicine
7/86-6/90	Residency, Radiation Oncology, Stanford University Hospital, Stanford, CA
7/90-8/90	Acting Assistant Professor (50%), Dept of Radiation Oncology, Stanford Univ School of Medicine
9/1/90-8/3197	Asst Professor, Department of Radiation Oncology, Stanford University School of Medicine
9/1/97-present	Assoc Professor, Department of Radiation Oncology, Stanford University School of Medicine Current responsibilities include: Laboratory and clinical research, teaching (fellows, residents, medical and graduate students), and patient care (general radiation oncology and protocol patient care).
9/1/02-Present	Advising Dean, School of Medicine

Other Experience and Professional Memberships

1989	American Cancer Society Clinical Oncology Fellowship
1988	American Society for Therapeutic Radiology and Oncology (ASTRO) Fellowship
2001-Present	Associate Editor for Radiation Research
2003-Present	Journal Clinical Oncology
2001-Present	Study Section Member: Clincial Oncology Study Section

Honors

1992, 1993	Lazard Faculty Scholar

Exhibit 1 -1

1991-1994 American Cancer Society Clinical Oncology Career Development Award

B. Selected peer-reviewed publications (in chronological order) (from a total of 98 published papers or papers in press)

Rupnow, B.A., Alarcon, R.M., Giaccia, A.J. and Knox, S.J. p 53 mediates apoptosis induced by c-Mcy activation in hypoxic or gamma irradiated fibroblasts. Cell Death and Diff. 5:141-147, 1998.

Ning, S.C., Shui, C., Khan, W.B., Benson, W., Lacey, D.L. and Knox, S.J. Effects of keratinocyte growth factor on the proliferation and radiation survival of human squamous cell carcinoma cell lines in vitro and in vivo. Int J Radiat Oncol Biol Phys 40: 177-187, 1998.

Rupnow, B.A., Murtha, A.D., Alarcon, R.M., Giaccia, A.J., Knox, S.J. Direct evidence that apoptosis enhances tumor responses to fractionated radiotherapy. Cancer Research 58: 1777-1784, 1998.

Rupnow, B.A. and Knox, S.J. The role of radiation-induced apoptosis as a determinant of tumor response to radiation therapy. Apoptosis 4: 1-29, 1999.

Ning, S.C. and Knox, S.J. G2/M Phase Arrest and Apoptotic Cell Death of HL60 Cells Irradiated with Exponentially Decreasing Low Dose Rate Gamma Irradiation Radiation Research 151:659-669, 1999.

Knox, S.J., Goris, M.L., Tempero, M., Weiden, P.L., Gentner, L., Breitz, H., Adams, G.P., Axworthy, D., Gaffigan,S., Bryan, K., Fisher, D.R., Colcher, D., Horak, I.D. and Weiner, L.M. Phase II Trial of Yttrium-90-DOTA-Biotin pretargeted by NR-LU-10 antibody/streptavidin in patients with metastatic colon cancer. Clinical Cancer Research 6:406-414, 2000.

Steinauer, K.K., Gibbs, I., Ning, S., French, J.N., Armstrong, J. and Knox, S.J. Radiation induces upregulation of cyclooxyginase-2 (cox-2) protein in PC-3 cells. Int J Rad Oncol Biol Physics. 48:325-328, 2000.

Williams, S.S., French, J.N., Gilbert, M., Rangaswami, A.A., Walleczek, J. and Knox, S.J. Bcl-2 overexpression results in enhanced capacitative calcium entry and resistance to SKF-96395 induced apoptosis. Cancer Research 60:4358-4361, 2000.

Armstrong, J.S., Steinauer, K.K., French, J., Killoran, P.L., Walleczek, J., Kochanski, K. and Knox, S.J. Bcl-2 inhibits apoptosis after mitochondrial "uncoupling" but does not prevent mitochondrial transmembrane depolarization. Experimental Cell Research. 262:170-9, 2001.

Ning, S., Laird, D., Cherrington, J.M., and Knox, S.J. Anti-angiogenic agents SU5416 and SU6668 potentiate antitumor effects of radiation therapy. Radiation Research 157:45-51, 2002.

Armstrong, J.S., Steinauer, K.K., Lecane, P., Birrell, G., Peehl, D.M. and Knox, S.J. Role of glutathione depletion and reactive oxygen species generation in apoptotic signaling in a human B lymphoma cell line. Cell Death and Differentiation 9:252-63, 2002.

Ning, S., Laird, D., Cherrington, J.M., and Knox, S.J. Anti-angiogenic agents SU5416 and SU6668 potentiate antitumor effects of radiation therapy. Radiation Research 157:45-51, 2002.

Li, L., Steinauer, K.K., Gibbs, I., Armstrong, J.S., **Knox, S.J.** Radiation-induced cyclooxygenase-2 upregulation is redox status dependent in prostate cancer cells. Radiation Research 160:617-621, 2003.

Li, L., Wartchow, C.A., Danthi, S.N., Shen, Z., DeChene, N., Pease, J., Choi, S., Doede, T., Chu, P., Ning, S., Lee, Y., Bednarski, M.D., **Knox, S.J.** ^{90}Y labeled integrin antagonist (1A-NP-^{90}Y) and anti-Flk-1 antibody (anti-Flk-1 MAB-NP-^{90}Y): novel anti-angiogenesis therapies using nanoparticles. Int J Radiation Oncology, Biology, Physics 58:1215-1227, 2004.

Davis, T.A., Kaminski, M.S., Leonard, J.P., Wahl, R., Kroll, S., Coleman, M., Goris, M., Levy, R., **Knox, S.J.** A randomized controlled trial of Tositumomab and ^{131}Iodine Tositumomab (Bexxar^{R}) versus Tositumomab for patients with relapsed or refractory low-grade or transformed low grade non-Hodgkin's lymphoma. In Press in Clinical Cancer Research.

Ning, S., **Knox, S.J.** Increased cure rate of glioblastoma using concurrent therapy with radiation and arsenic trioxide. Int J Radiation Oncology, Biology, Physics 60(1):197-203, 2004.

Exhibit 1 -2

Husbeck, B., Nonn, L., Peehl, D.M., **Knox, S.J.** Tumor-selective killing by selenite in patient-matched pairs of normal and malignant prostate cells. Prostate. 66(2):218-225, 2006

Ning, S., Hartley, C., Molinvex, G., **Knox, S.J.** Darbepoietin alfa (Aranesp) potentiates the efficacy of radiation therapy in vivo. In Press in Cancer Research.

Ning, S., **Knox, S.J.** Increased cure rate of glioblastoma using concurrent therapy with radiation and arsenic trioxide. Int J Radiation Oncology, Biology, Physics 60(1):197-203, 2004.

Davis, T.A., Kaminski, M.S., Leonard, J.P., Hsu, F.J., Wilkinson, M., Zelenetz, A., Wahl, R.L., Kroll, S., Coleman, M., Goris, M., Levy, R. and **Knox SJ.** The radioisotope contributes significantly to the activity of radioimmunotherapy. Clin Cancer Res 10(23):7792-7798, 2004.

Husbeck, B., Peehl, D.M., **Knox, S.J.** Redox modulation of human prostate carcinoma cells by selenite increases radiation-induced cell killing. Free Radic Biol Med 38(1):50-57, 2005.

Ning, S., Hartley, C., Molinvex, G., **Knox, S.J.** Darbepoietin alfa potentiates the efficacy of radiation therapy in mice with corrected or uncorrected anemia. Cancer Res 65(1):284-290, 2005.

Rosenberg, A. and **Knox, S.J.** Radiation sensitization with redox modulators: A promising approach. Int J Radiat Oncol Biol Phys 64(2):343-354, 2006.

Patel, D.A., Kochanski, J., Suen, J., Fajardo, A.W., Hancock, S.L., and **Knox, S.J.** Clinical manifestations of noncoronary atherosclerotic vascular disease after moderate dose irradiaiton. Cancer. 106:718-725, 2006.

Ning, S. and **Knox, S.J.** Optimization of combination therapy of arsenic trioxide and fractionated radiotherapy for malignant glioma. Int J Radiat Onc Biol Phys 65(2):493-498, 2006.

Husbeck, B., Bhattacharyya, R., Feldman, D. and **Knox, S.J.** Inhibition of androgen receptor signaling by selenite and methylseleninic acid in prostate cancer cells: two distinct mechanisms of action. Mol Cancer Ther. 5(8):2078-2085, 2006.

Street, H.H., Goris, M.L., Fisher, G.A., Wessels, B.W., Cho, C., Hernandez, C., Zhu, H.J., Zhang, T., Nangiana, J.S., Shan, J.S., Roberts, K. and **Knox, S.J.** Phase I study of 131I-chimeric(ch) TNT-1/B monoclonal antibody for the treatment of advanced colon cancer. Cancer Biother Radiopharm. 21(3):243-56, 2006.

Kim, Y.H., Duvic, M., Obitz, E., Gniadecki, R., Iversen, L., Osterborg, A., Whittaker, S., Illidge, T.M., Schwartz, T., Kaufmann, R., Cooper, K., Knudsen, K.M., Lisby, S., Baadsgaard, O., **Knox, S.J.** Clinical efficacy of zanolimumab (HuMax-CD4): two Phase 2 studies in refractory cutaneous T-cell lympoma, Blood. 109:4655-4622, 2007.

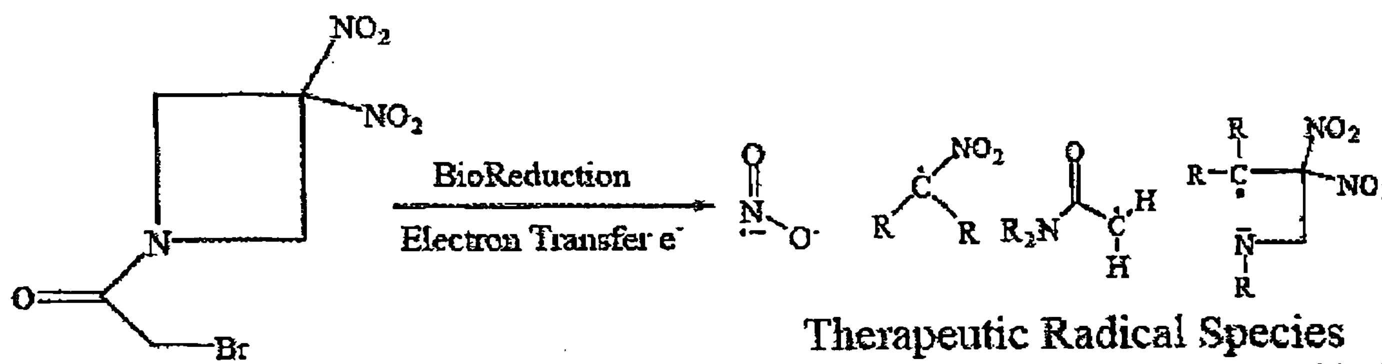
Ning, S., Chen, Z., Dirks, A., Hsu, M., Husbeck, B., O'Neill, M., Bedogni, B., Powell, M.A., **Knox, S.J.** Targeting the Ingegrin $\alpha_v\beta_3$ and AKT-mediated signal transduction pathways to enhance radiation-induced anti-angiogenesis. Radiation Research. 168:125-133, 2007.

Sinclair, A.M., Todd, M.D., Forsythe, K., **Knox, S.J.**, Elliot, S., Begley, G. Expression and function of erythropoietin receptors in tumors: implications for the use of erythropoiesis-stimulating agents in cancer patients. Cancer. 110(3):477-488, 2007.

Sgouros, G., **Knox, S.J.**, Joiner, M.D., Morgan, W.F., Kassis, A.I., Mird, C.E.: Bystander and Low Dose Rate Effects - Are these relevant to radionuclide therapy. J Nuclear Medicine. 48(10):1683-91, 2007.

Exhibit 1 -3

Decomposition of ABDNAZ by Bioreduction
Hypoxic Region with Low Oxygenation



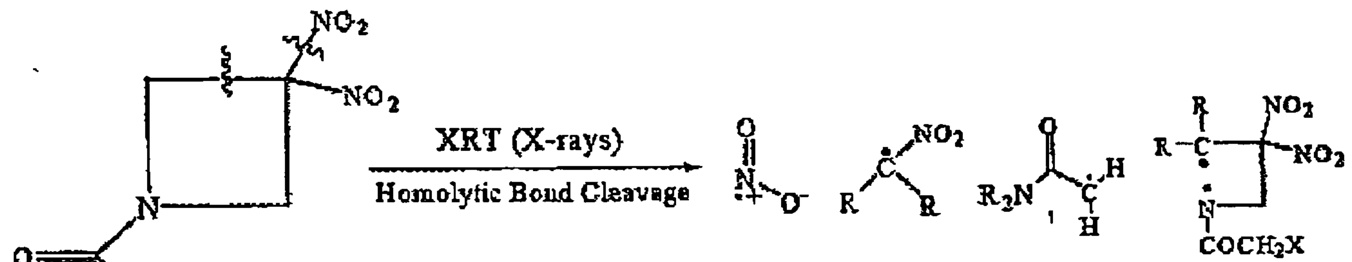
Therapeutic Radical Species
(Reacts with DNA, Lipids and other critical cellular components leading to cell death)

Ammonia CO_2 NO N_2 Acetic Acid

Non-Toxic By Products

Exhibit 2

Decomposition of ABDNAZ by Clinical XRT



1. Direct Therapeutic Radical Species
(Reacts with Lipids, DNA and other essential cellular components resulting in cell death)

$\oplus$ O_2 | H_2O

2. Indirect Therapeutic Radical Species
(Reacts with Oxygen and Water resulting in conventional ROS mediated cell death)

$O_2^{\cdot-}$ $HO^{\bullet}$ HOOH

Exhibit 3

ABDNAZ kills Tumor Cells in Hypoxic Regions

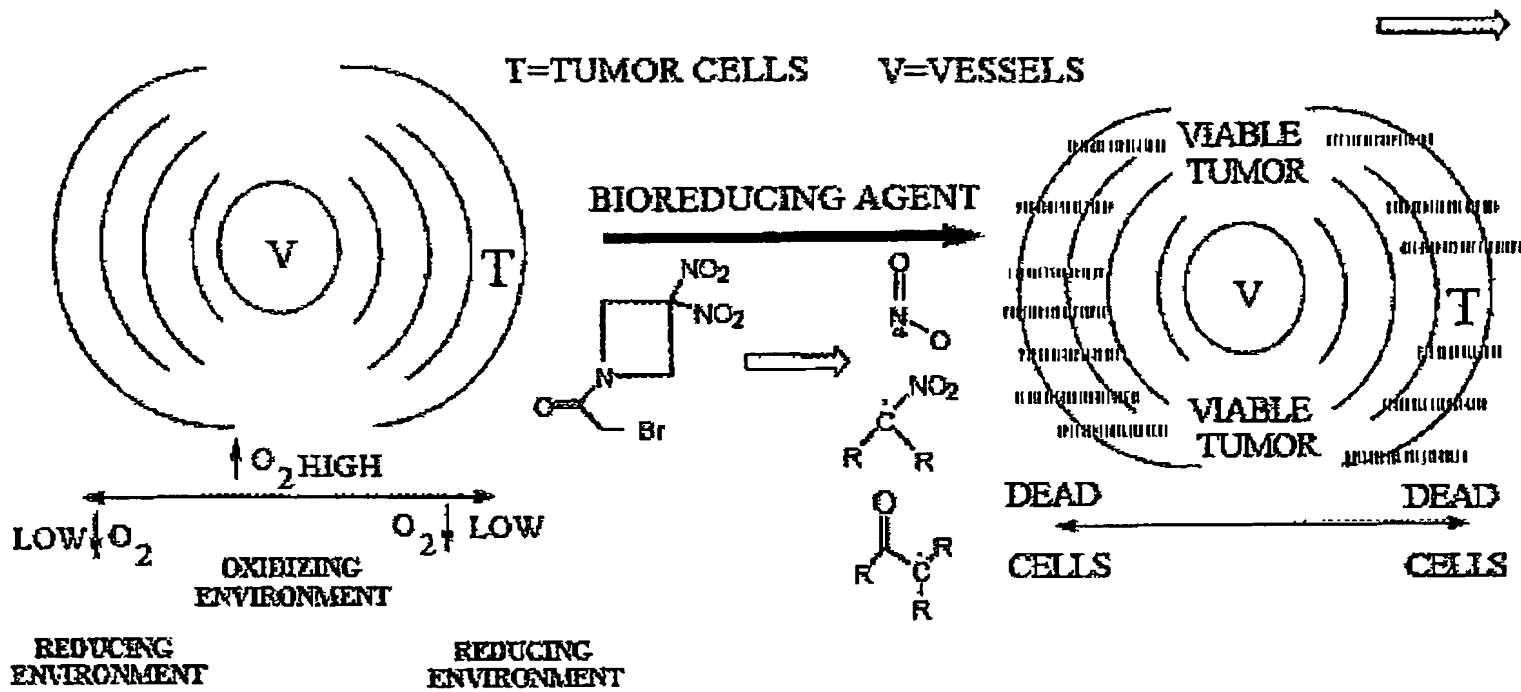


Exhibit 4

Application No. 10/681,855
Filed October 7, 2003

Attorney Docket No. 067425-5001-US

ABDNAZ Is a Potent and Selective Killer of Hypoxic Tumor Cells

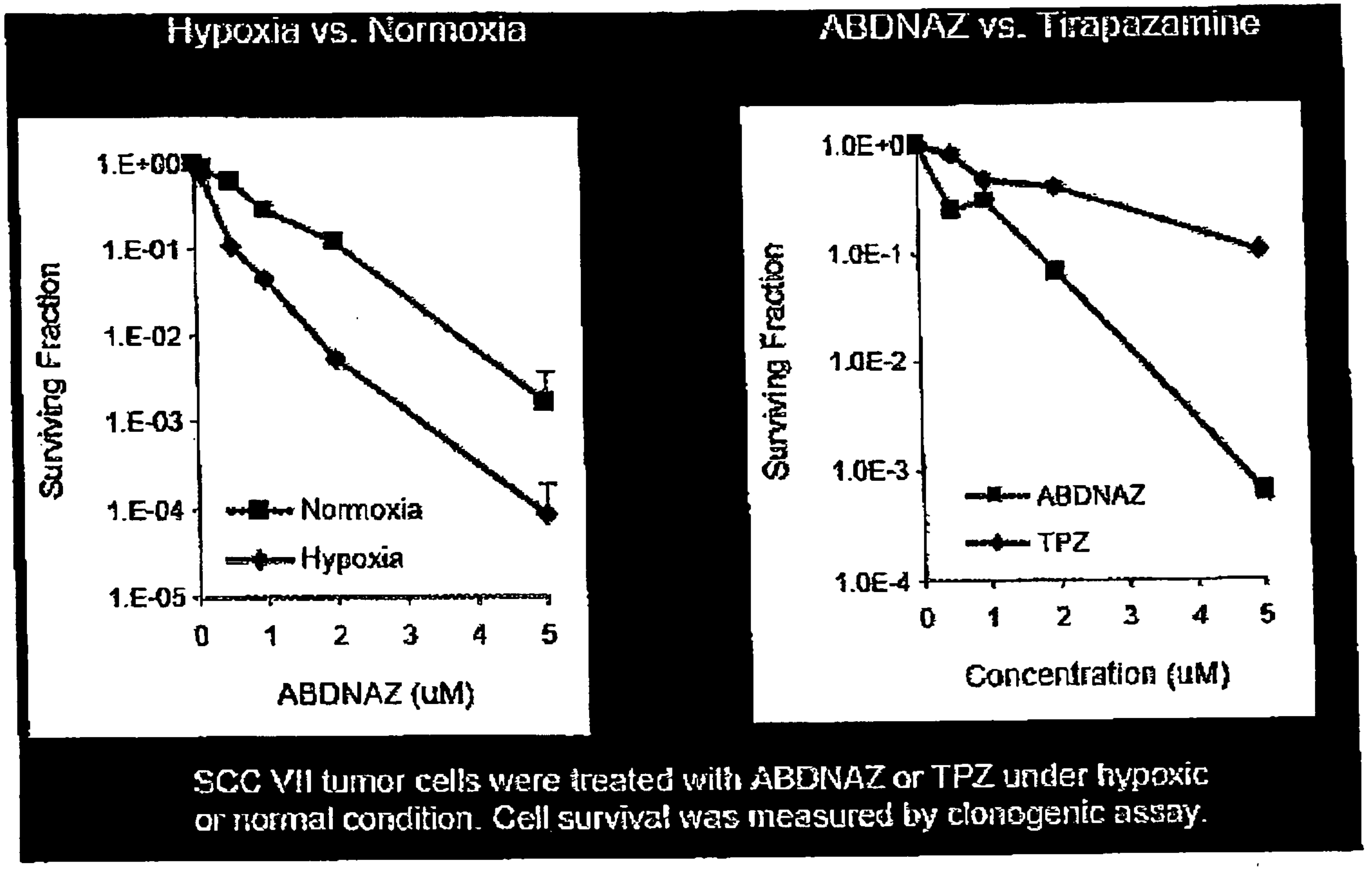


Exhibit 5